was found to be an inhibitor of the enzyme subsequent to the crystallization. However at present no evidence suggests that the inactivation is due to the oxidation of the active site cysteine sulfenic acid.
The structures give insight into the determinants of the specificity of the enzyme, suggesting that βphe55 and βphe55 obstruct access to the cysteine claw complex for larger substrates allowing the hydration of only smaller aliphatic nitriles.

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Crystal Structure Of Peroxide-bound Manganese Superoxide Dismutase
Gloria Borgstahl, Jason Porta.
University of Nebraska Medical Center, Omaha, NE, USA.
The superoxide dismutase (SOD) enzymes are important antioxidant agents that protect the cells from reactive oxygen species (ROS). The SOD family is responsible for catalyzing the disproportionation of superoxide to oxygen and hydrogen peroxide. We report the first ever structure of a superoxide dismutase with bound hydrogen peroxide in the active site. Synchrotron X-ray diffraction data was collected from *Escherichia Coli* MnSOD crystals that were soaked in a cryosolution containing 0.008%(v/v) hydrogen peroxide and cryocooled to 100K. Structural refinement to 1.55 Å and close inspection of the active site revealed electron density for hydrogen peroxide in three of the four active sites. The hydrogen peroxide molecules are sideways bonded to the manganese in the position normally assumed by water or inhibitory hydroxide. The hydrogen peroxide molecules are present in active sites B, C and D. It was observed that MnSOD enzymes could accommodate two hydrogen peroxide molecules per active site in an antiprismo coordination geometry. Comparison of the peroxide-bound active site with the wild-type trigonal bipyramidal form shows a shifting of the gateway residues Tyr34 and His30, thereby preventing the escape of the bound ligands. The peroxide-bound form more closely resembles the active-site geometry of six-coordinate octahedral form (1D5N), where hydroxide ligands were trapped in the active site by cryocooling.

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Characterization of the Monomer-Dimer Equilibrium of Recombinant Histo-aspartic Protease from Plasmodium falciparum
Huogen Xiao[1], Lee-Ann Briere[2], Stanley D. Dunn[2], Rickey Y. Yada[1].
[1]University of Guelph, Guelph, ON, Canada, [2]University of Western Ontario, London, ON, Canada.
Malaria is a devastating disease that infects and kills 1-2 million people annually. Histo-aspartic protease (HAP) from *Plasmodium falciparum*, the most lethal of all Plasmodium parasites, is an intriguing aspartic protease due to its unique structure and its potential as an antimalarial target. Substantial effort has been devoted to investigate the structure function of this protease. The present study investigated the molecular state of HAP as related to enzymatic activity. Gel filtration chromatography indicated that recombinant Trx-tHAP fusion protein aggregated during purification and that aggregation could be prevented through the addition of 0.2% CHAPS. Using this latter technique as well as sedimentation velocity and sedimentation equilibrium ultracentrifugation, it was shown that the recombinant mature HAP (mtHAP), in which the His-tag, thioredoxin and prosegement were removed, exists in a dynamic monomer-dimer equilibrium in solution and the dissociation constant is 20-30 μM. Enzymatic activity data also indicated that HAP was most active as a monomer. The monomeric form of mtHAP showed a Km of 9.7 μM and a turnover number, kcat, of 0.044s-1 on the internally quenched fluorescent synthetic peptide substrate EDANS-CO-CH2-CH2-CO-Ala-Leu-Glu-Arg-Met-Phe-Leu-Ser-Phe-Pro-Dap-(DABCYL)-OH (2837b) at pH 6.5. Inhibition studies showed that the activity of mtHAP was completely inhibited by 1 mM PMSF and to a lesser degree by 10 μM ALLN, 10 mM EDTA and 10 mM 1,10-phenanthroline, and was inhibited strongly by ZnCl2 and to a lesser extent by NaCl and KBr. The effects of temperature and salts on the monomer-dimer equilibrium of mtHAP were also investigated by using sedimentation equilibrium ultracentrifugation.

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Analysis of Monomeric and Dimeric Phosphorylated Forms of PKR
Christine Quartararo, Eric Anderson, Raymond Brown, Yu Shi, Xudong Yao, James Cole.
University of Connecticut, Storrs, CT, USA.
PKR (protein kinase R) is induced by interferon and is a key component of the innate immunity antiviral pathway. Upon binding dsRNA or dimerization in the absence of dsRNA, PKR undergoes autophosphorylation at multiple serines and threonines that activate the kinase. Although phosphorylation is known to enhance PKR dimerization, gel filtration analysis reveals a second, monomeric phosphorylated form. The monomeric and dimeric forms do not interconvert. The monomeric form dimerizes weakly with a Kd similar to unphosphorylated PKR. Isoelectric focusing and mass spectroscopy reveal that both the monomeric and dimeric forms are heterogeneous in their phosphorylation state. Equilibrium chemical denaturation analysis indicates that phosphorylation destabilizes the kinase domain by about 1.5 kcal/mol in the dimeric form but not in the monomeric form. Limited proteolysis also reveals that phosphorylation induces a conformational change in the dimeric form that is not detected in the monomeric fraction. The monomeric phosphorylated form binds dsRNA similarly to unphosphorylated PKR but the affinity is greatly reduced for the dimeric form. Despite these differences in biophysical properties, both phosphorylated forms are catalytically competent and are activated to phosphorylate the PKR substrate eIF2a in the absence of dsRNA. Thus, both monomeric and dimeric forms of phosphorylated PKR may participate in the interferon antiviral pathway.

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Structural Studies of Enzymatic Hydrolysis of Cellulose by Neutron Scattering and Reflectivity
Michael S. Kent[1], Jaclyn K. Murton[1], Elizabeth L. Carles[1], Frank Zendejas[2], Rex Hjelm[3], Bulent Akgun[4], James Browning[5], John Ankner[5], Jacob Urquidi[6], Blake Simmons[2].
[1]Sandia National Labs, Albuquerque, NM, USA, [2]Sandia National Labs, Livermore, CA, USA, [3]Los Alamos National Labs, Los Alamos, NM, USA, [4]NIST, Gaithersburg, MD, USA, [5]Oak Ridge National Labs, Oak Ridge, TN, USA, [6]New Mexico State University, Las Cruces, NM, USA.
Improving the efficiency of enzymatic hydrolysis of cellulose is a key technological hurdle in reducing the cost of producing ethanol from lignocellulosic material. Typically, enzymatic hydrolysis proceeds to only a limited extent, high solution-to-solids ratios are required, and the rate of enzymatic hydrolysis typically decreases with time. A range of mechanisms have been proposed to explain these phenomena including product inhibition, denaturation of enzymes, nonproductive binding, and many others. We are studying the interaction of enzymes with cellulose to help unravel these mechanisms. Our studies include UV absorption and circular dichroism of enzymes in solution, small angle neutron and X-ray scattering (SANS, SAXS) of cellulose during hydrolysis, and neutron reflectivity (NR) of enzymes interacting with model cellulose surfaces. Insight from these studies should aid the development of more efficient enzyme systems and pretreatments.

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Expression And Purification Of A Stable, Monomeric Creatine Kinase
Gregg G. Hoffman, W. Ross Ellington.
Florida State University, Tallahassee, FL, USA.
Three isoform gene families of creatine kinase (CK) are present in animals. Two of these, mitochondrial and cytoplasmic CKs, are obligate oligomers . There is substantial evidence for functional interaction between subunits. Attempts at generating active, monomeric CKs have failed or in one case produced ephemerally active but unstable monomers. A third CK isoform, the so-called flagellar CK, is monomeric but is composed of three complete, contiguous CK domains. Each of these domains is catalytically competent but there is clear interaction between active sites (Hoffman et al., FEBS J 275: 646-654 [2008]). In the present effort, we have used a flagellar CK expression construct as a platform to engineer, express and purify a single domain, monomeric CK. Boundaries between the three domains (D1, D2 and D3) were identified by comparison of key catalytic residues and predicted secondary structural elements. A cDNA coding for D2 was amplified by PCR and inserted into an expression vector. Subsequent expression and purification yielded a recombinant CK which was stable as evidenced by the retention of activity over several weeks. Size exclusion chromatography showed that this CK was monomeric as expected, with a mass similar to the predicted Mr based on the amino acid composition. The engineering of a monomeric CK in the present effort clearly shows that oligomerization is not required for catalysis. Conventional wisdom supports the view that CKs evolved from a related phosphotransferase, arginine kinase (AK). AKs are typically monomeric. It seems likely that oligomerization occurred later in the evolution of CKs perhaps due to the selective pressure for targeting to and binding in intracellular compartments. (Supported by NSF grant IOB-0542236 to WRE).

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Role of Rim Tyr/Trp Residues in Interfacial Activation of Phospholipase C Enzymes
Su Guo, Mary F. Roberts.
Boston College, Chestnut Hill, MA, USA.
The *Bacillus thuringiensis* phosphatidylinositol-specific phospholipase C (btPI-PLC) as a model system and mammalian PI-PLCδ1 without an intact PH domain have been examined for the contribution of rim aromatic groups to protein binding to vesicles and the correlation of this with catalytic activity. In the bacterial enzyme, two tryptophan residues (Trp47 in the two-turn helix B and Trp242 in a disordered loop) are critical for binding to interfaces; of the many several Tyr residues mutated, replacement with alanine at several sites (close to helix B as well as the active site) weakens membrane binding. For many of these residues the loss in binding affinity approximates what is